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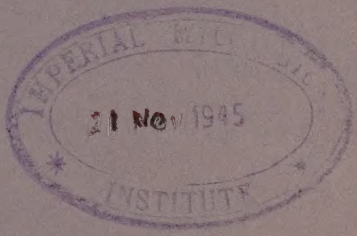

Charcoal Rot of Corn and Sorghum

J. E. Livingston
Department of Plant Pathology

LINCOLN, NEBRASKA
APRIL 1945

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CONTENTS

Introduction 3

Symptoms of Charcoal Rot 4

The Effect of Temperature, Carbohydrate Source, Nitrogen Source, and H-ion on the Growth of *Macrophomina phaseoli* in Culture 7

- Temperature 7
- Carbohydrate Source 9
- Nitrogen 11
- H-ion Concentration 12
- Changes in pH of Medium During Growth 13

The Effect of Soil Temperature and Soil Moisture on the Infection of the Grain Sorghum Variety Alliance by *M. phaseoli* 14

- Relation of Soil Temperature with High and Low Soil Moisture to Infection of Grain Sorghum Plants 14
- Relation of Soil Temperature to Infection of Grain Sorghum in Steam-Sterilized and Chloropicrin-Treated Soil at a Uniform Soil Moisture 18
- Relation of Soil Moisture to Infection in Soil at a Uniform Temperature 22
- Variation in the Susceptibility of Sorghum Varieties to Charcoal Rot 25
- Effect of Crop Rotation on the Development of Charcoal Rot 26
- Method of Infection 27
- Discussion 28
- Summary 30
- Literature Cited 31

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Charcoal Rot of Corn and Sorghum

J. E. LIVINGSTON[1]

INTRODUCTION

ALTHOUGH charcoal rot, caused by *Macrophomina phaseoli* (Maubl.) Ashby, is an old disease, its importance as a source of damage to corn and sorghum, and particularly to corn, was not recognized in the Midwestern cornbelt states until the late 1930's, after several years of severe drouth.

FIG. 1.—Lodging of sorghum caused by the charcoal rot organism.

In Nebraska, the disease caused considerable damage to the 1940 grain sorghum crop in the south central counties, and the following year it caused serious lodging (Fig. 1), and reduction of yield in both corn and sorghum in all sections of the state. Lodging was particularly serious with the grain sorghums because heads from lodged plants could not be harvested with a combine. Premature ripening and lodging of corn occurred earlier and in a higher percentage of the stalks than with sorghum.

Field observations indicated that some varieties of sorghum were damaged less than others; thus it appeared desirable to test thoroughly all varieties to determine whether any were sufficiently resistant to be recommended for field planting or to be used in a breeding program.

[1] Assistant Plant Pathologist, Nebraska Agricultural Experiment Station.

Very little information was available on the susceptibility of varieties or the conditions favoring infection. Uppal et al. (*25*), working in India, published the only critical study of the conditions affecting the development of charcoal rot in sorghum. These authors studied the effect of temperature and moisture on infection of sorghum in soil that was sterilized before inoculation and found that the high soil temperature of 35.5°C. was the optimum for the development of the disease and that no infection was obtained at or below 50 per cent of the water-holding capacity of the soil. These findings did not agree completely with field observations in Nebraska where the disease was most severe under conditions of low moisture.

It seemed desirable therefore to study the influence of soil environment on infection and the development of stalk rot before attempting an extensive varietal testing program. Such information would also serve as a guide in predicting the geographic distribution of the disease and the severity of the stalk rot in a given season.

Symptoms of Charcoal Rot

The charcoal rot organism caused both a root rot and a stalk rot of sorghum and corn when grown in inoculated soil in the greenhouse. The root rot symptoms on corn, as described by Semeniuk (*17*), are similar to those on sorghum. Blighted seedlings, as a result of root infection, first showed a drying of the tips of the lower leaves. This drying progressed, but the entire plant usually turned yellow and rotted off at or just below the ground line before any but the lowest leaves became completely dry. The plants appeared to be suffering from lack of moisture; however, the addition of water did not bring about recovery. This was because the primary roots had rotted away, the secondary roots either had not formed or had been destroyed, and the mesocotyl was black and shrivelled throughout most of its length, as shown in Fig. 2.

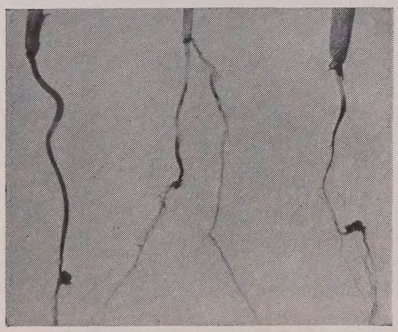

FIG. 2.—Charcoal rot infection of the mesocotyl.

Less severely diseased seedlings often were able to initiate and establish secondary roots. When these were established the seedling had a chance of survival, especially if the soil was moist. The secondary roots also became infected, as shown in Fig. 3, but they were not so easily destroyed as were the primary roots.

The nature of the root rot on sorghum seedlings is shown in Fig. 4. The rotting varied from a few scattered lesions to a wet, black rot of the entire root. Root lesions, whether on primary or secondary roots, were

FIG. 3.—Lesions on adventitious roots of sorghum plants grown in wet soil.

brown and watersoaked at first but soon became dark brown or black. They appeared first and most frequently near the base of the roots, less frequently near the tip, with many lesions also at scattered places over the root. Girdling took place very soon after infection, after which the rot developed up and down the root. The rot was primarily cortical and often extended several inches along the root before the central portion was attacked. The root continued functioning until the stele was attacked; after that it died and shriveled.

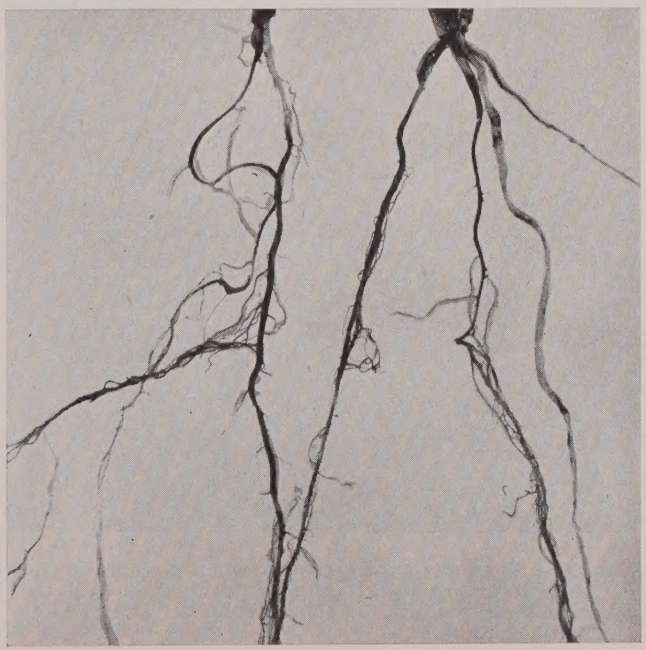

FIG. 4.—Lesions on adventitious roots of sorghum plants grown in dry soil.

The stalk rot symptoms in mature sorghum in the field are nearly identical to those described by Mackie (*13*) for corn. Premature ripening is not so evident in sorghum as in corn. Usually lodging of the stalk is the first indication of the disease. Sometimes the outside of the stalk shows a dark brown discoloration, but more often it is slightly lighter colored than normal. In some varieties of sorghum, diseased stalks are dry and brittle, whereas in other varieties the stalks are soft and spongy. When mature diseased stalks are split, there is usually but little discoloration. The pith can be found in all degrees of disintegration, and the pith and fibers are covered with the small black sclerotia of the fungus, giving the appearance of having been dusted with charcoal (Fig. 5).

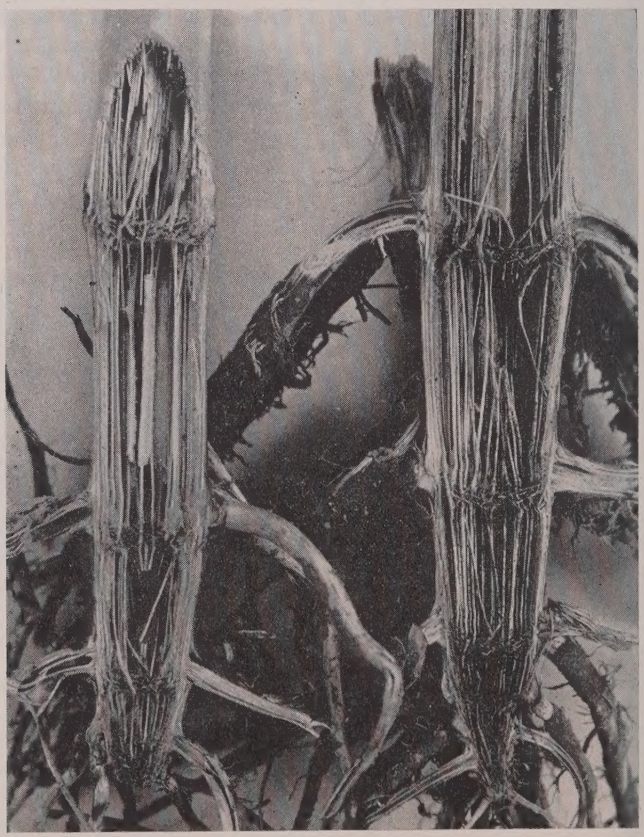

FIG. 5.—Charcoal rot of corn showing disintegration of the pith in the lower portion of the stalk and the presence of sclerotia on the vascular strands and remaining pith.

In the field, shredding of the inside of the stalk and sclerotial formation usually are confined to the lower 6 to 10 inches of the stalk. However, in the greenhouse, sclerotia have occasionally been found all the way up to the head in sorghum and up to the tassel in corn.

The Effect of Temperature, Carbohydrate Source, Nitrogen Source, and H-ion on the Growth of *Macrophomina phaseoli* in Culture

Very little information is available on the growth of isolates of *M. phaseoli* from sorghum in pure culture. Uppal et al. (*25*) studied the effect of temperature and H-ion, but no results have been reported on the effect of different nutrients. The following experiments were designed to give a better understanding of the nutritional requirements and certain other factors affecting the growth of the sorghum isolate. In three of the tests isolates from corn, sugar beet, and catalpa also were included to determine whether differences in the growth of the various isolates might be such as to indicate the existence of physiological strains of the organism.

The isolate from sorghum was obtained by plating a single sclerotium from an infected stalk of Early Kalo on potato-dextrose agar. The isolate from corn was obtained in a similar manner. The isolates from sugar beet and catalpa were obtained by making tissue cultures from diseased seedlings of the respective hosts.

The cultural studies were made on a synthetic medium consisting of 5 gr. sodium chloride, 0.2 gr. magnesium sulfate, 0.1 gr. calcium chloride, 1 gr. mono-basic potassium phosphate, 1 gr. di-basic potassium phosphate, 10 gr. dextrose, 1.5 gr. peptone, and 17 gr. agar per liter of distilled water. The pH was adjusted to 7.0. Twenty cc. of the agar was poured into sterilized pyrex Petri dishes of uniform size. The plates were inoculated with a 5 mm. disc of inoculum from a 4-day-old culture growing on the synthetic medium and the growth rates were determined on the basis of the diameter of the colony after 3 days. Two measurements per plate were made at right angles to each other and the average recorded. The tests were conducted at 30°C. except in the temperature experiment.

Temperature

Uppal et al. (*25*) concluded from their experimental evidence that the optimum temperature for growth of the Broach strain of the organism was between 30° and 34.5°C. and between 30° and 37°C. for the Mohol strain. Growth was observed at temperatures as low as 9° and 11°, respectively, and as high as 40°C.

Kendrick (*11*) observed that an isolate from beans grew very slowly at 7.8° and 40° and that 30°C. was optimum for growth and for rapidity of sclerotial formation.

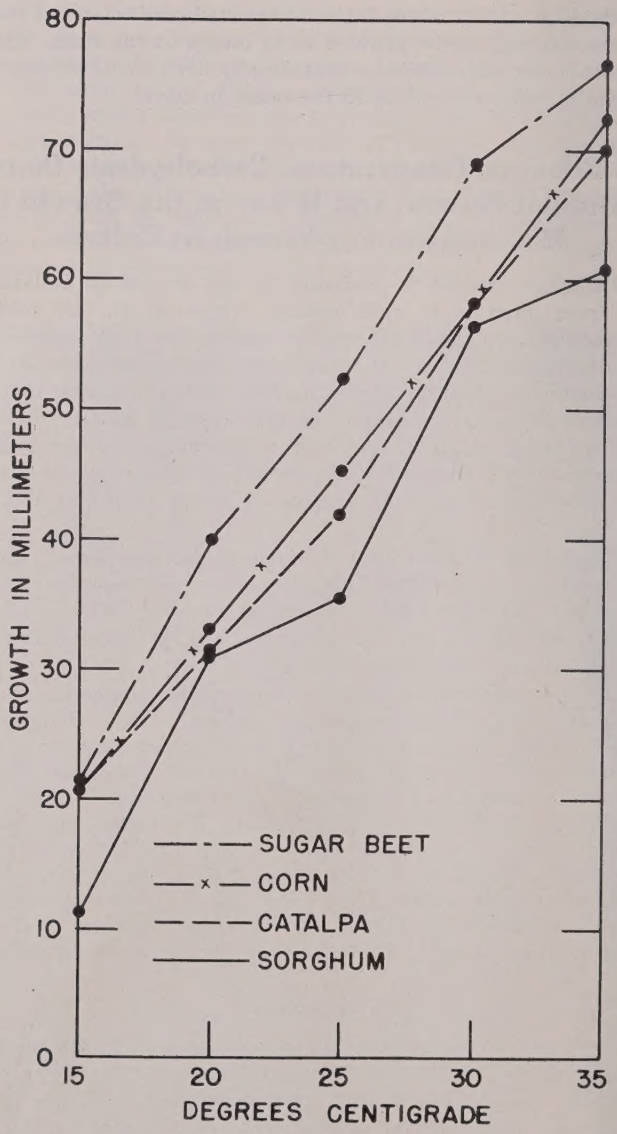

FIG. 6.—Total growth made by four isolates of *M. phaseoli* in three days when grown on synthetic agar media at different temperatures.

The data presented in Fig. 6 conform with those presented by Uppal et al. (25). However, the optimum temperature for growth was somewhat higher than that obtained by Kendrick (11) for the isolate from beans. Each of the four isolates grew best at 35°C., the highest temperature used. The isolate from sorghum made the least growth at all temperatures while the isolate from sugar beet grew most rapidly. The isolates from corn and catalpa grew at about the same rate.

Sclerotial formation occurred within 24 hours at 30° and 35°C. but did not appear until the third day at 15°C. Their appearance was apparently related to the rate of growth.

Carbohydrate Source

The effect of various forms of carbohydrate on the growth of the isolate of *M. phaseoli* from sorghum has not been reported in the literature.

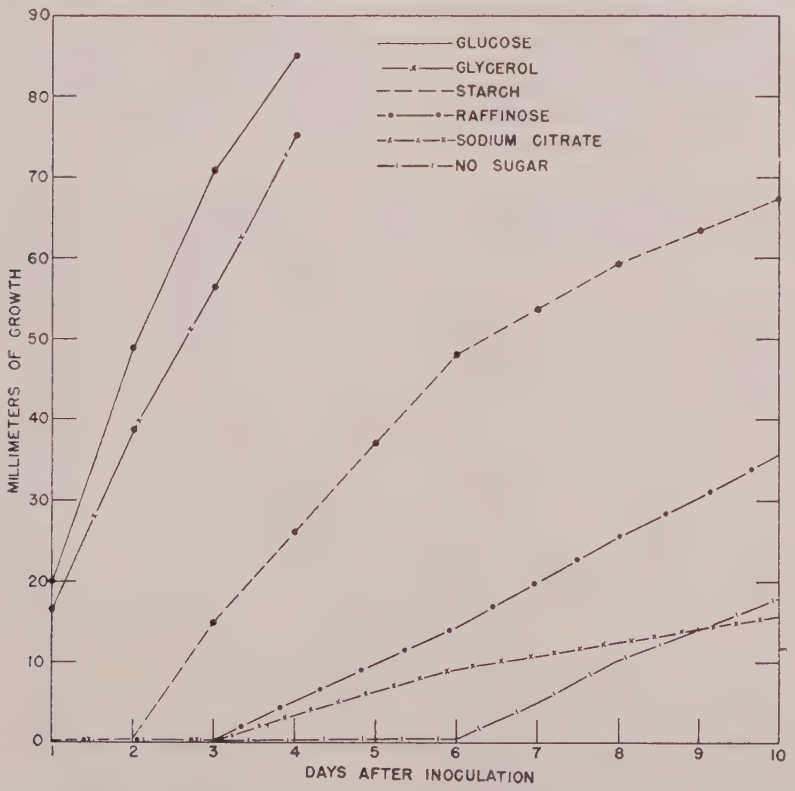

FIG. 7.—Average daily growth of *M. phaseoli* from sorghum on synthetic agar media containing different carbohydrates in a one per cent concentration by weight. Growth on fructose, mannose, galactose, and sucrose agar was similar to that on glucose and growth on maltose was similar to that on glycerol.

This problem was studied by substituting various carbohydrates for the dextrose in the synthetic medium. Raffinose, maltose, galactose, mannose, and fructose were used at the rate of 1 per cent by weight; and glucose, sucrose, glycerol, starch, and sodium citrate were used at the rates of 1 and 2 per cent by weight.

Growth of the sorghum isolate on agar containing the various carbohydrates at a concentration of 1 per cent is shown in Fig. 7. Fructose supported the best growth, with glucose, mannose, galactose, and sucrose being nearly as good. Very slow growth was obtained on the media containing raffinose and sodium citrate, whereas the growth on starch agar was a little better. No growth was visible until the sixth day on media to which no carbohydrates had been added.

The effect of the materials used at two concentrations is shown in Table 1. Less than one-third as much growth was obtained with the 2 per cent concentration of sodium citrate as with the 1 per cent concentration. In the case of starch the effect was the opposite, much better growth being obtained with a 2 per cent concentration. Sucrose supported somewhat better growth in a concentration of 2 per cent, whereas with glucose and glycerol there was only a small difference in the amount of growth obtained with the two concentrations.

Table 1.—*Effect of different sources of carbohydrate on mycelial growth and sclerotial formation of M. phaseoli on a synthetic agar medium*

Sugars	Grams per liter	Diameter of colony after 3 days	First appearance of sclerotia[1]	Sclerotia after 5 days
	gms.	*mm.*	*days*	
Sodium citrate	10	1.8	10	none
Sodium citrate	20	0.5	none	none
Starch	10	15.1	10	none
Starch	20	50.2	4	many
Glycerol	10	56.6	4	many
Glycerol	20	54.9	4	many
Sucrose	10	61.7	4	many
Sucrose	20	70.2	3	many
Glucose	10	71.2	3	many
Glucose	20	72.9	4	many
Raffinose	10	T	none	none
Maltose	10	52.1	3	many
Galactose	10	67.1	5	few
Mannose	10	69.8	3	many
Fructose	10	73.1	4	many
No sugar	0	T	none	none

[1] Culture observed for 16 days.

With one exception sclerotia appeared most rapidly in those plates supporting the best mycelial growth. The fungus on galactose agar produced only a few sclerotia, and these were slow in appearing.

Nitrogen

The effect of nitrogen on the rate of growth of the four isolates of the charcoal rot organism was studied by adding peptone, asparagine, urea, ammonium nitrate, sodium nitrate, and calcium nitrate to the synthetic agar medium at the rate of 1.5 grams per liter. The growth curve was the same for all isolates on the various nitrogen media (Fig. 8). However, there were marked differences in the amount of growth obtained after three days.

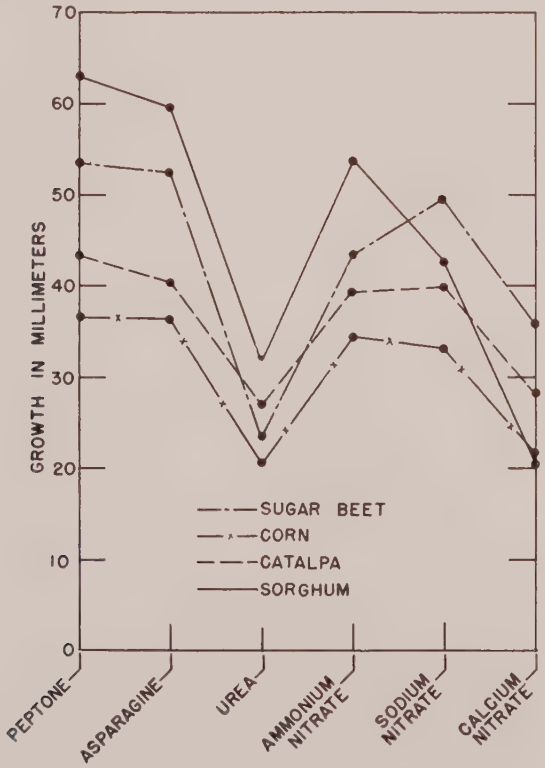

FIG. 8.—Total growth made by four isolates of *M. phaseoli* in three days on synthetic agar media containing different sources of nitrogen.

Peptone was the best nitrogen source, followed closely by asparagine. The isolates from corn and sorghum preferred ammonium nitrate to sodium nitrate, but with the isolates from sugar beet and catalpa the reverse was true. Urea supported the poorest growth with all the isolates except the one from sorghum, in which case calcium nitrate was poorest.

There were some noticeable differences in the formation of sclerotia by the isolates on the various nitrogen compounds. The sclerotia of the sorghum and corn isolates were concentrated in the center of the plates containing urea, ammonium nitrate, and calcium nitrate, whereas on the other media they were scattered throughout the mycelial mat. With the catalpa and sugar beet isolates the sclerotia were scattered throughout the mycelial mat on all the media.

The sclerotia of the sorghum isolate were very small and tended to group in rays radiating from the point of inoculation at the center of the plate. This type of grouping was not noted with the other isolates. The corn and catalpa isolates produced more and larger sclerotia than the sorghum isolate on all media. The sugar beet isolate was similar to the isolates from catalpa and corn with two exceptions. With ammonium nitrate a relatively small number of sclerotia were produced and with urea the sclerotia were very small and very numerous. The difference in the amount of growth made by the different isolates on the various nitrogen media and the differences in the formation of sclerotia indicate that there are physiological differences in the isolates.

H-ion Concentration

The effect of H-ion concentration on the growth of the charcoal rot organism has been studied by Uppal et al. (25). These authors reported that in nutrient broth the optimum growth of the Broach and Mohol strains occurred between pH 3.4 and 6.4 with a very decided drop between 3.4 and 2.6. The rate of growth tapered off more or less gradually from 6.4 to 12.4. On Dox's agar the optimum reaction for the two strains was also between 3.4 and 6.4. However, this difference in rate of growth largely disappeared after 96 hours because of the progressive change toward acidity of the more alkaline media.

The results presented in Fig. 9 were obtained by growing the isolates of *M. phaseoli* from sorghum, corn, sugar beet, and catalpa on synthetic agar media that had been adjusted to the desired pH by adding N/10 sodium hydroxide or N/10 hydrochloric acid. The amount of growth obtained with the four isolates at the different concentrations of H-ion varied considerably. The sugar beet isolate grew very well on media with pH readings from 5.0 to 8.0. The corn isolate grew well on media with pH readings from 5.0 to 7.0 but growth dropped off considerably at 8.0. The catalpa isolate grew least and the sorghum isolate best on media at pH 5.0. All the isolates except the one from catalpa made their poorest growth on media at pH 8.0. Optimum growth occurred between 6.0 and 7.0 with all except the sorghum isolate.

The sorghum isolate did not respond to pH as did the others. The best growth was obtained on media with a pH reading of 5.0 with very little difference in the amount of growth on the media at the other three H-ion concentrations. The media at pH 5.0 was slightly soft, a condition that may have had an influence on the growth of the sorghum isolate.

These results are in agreement with those obtained by Uppal et al. (25) in India who found that charcoal rot isolates grew over a wide pH range with a more or less gradual tapering off of growth in the more alkaline media.

Changes in pH of Medium During Growth

The study of the growth of the charcoal rot isolates on media with different concentrations of hydrogen ions and on media with different sources of nitrogen was terminated at the end of the third day. Changes in the pH of the media brought about by the growth of the organism were determined by melting the agar, removing the mycelial mat, and testing the melted agar electrometrically.

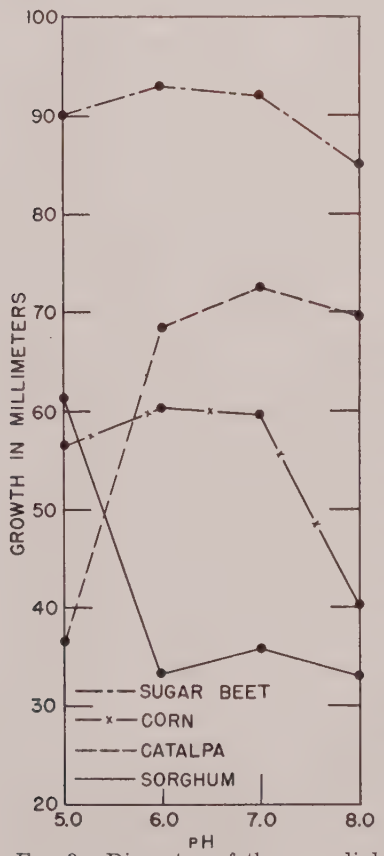

FIG. 9.—Diameter of the mycelial mat of four isolates of *M. phaseoli* when grown for three days on synthetic agar media of different hydrogen ion concentrations.

Results presented in Table 2 indicate that the charcoal rot isolates tend to change the pH of the medium toward neutrality, although there was some difference in the ability of the various isolates to bring about these changes. The corn isolate was most active in lowering the pH, bringing it from 8.0 down to 6.9, and the sugar beet isolate raised the pH the most, from 5.1 to 5.9.

There were also some differences in the changes produced by the isolates in the pH of the media with different sources of nitrogen. The sugar beet isolate produced the greatest changes, raising the pH of all the media except that containing ammonium nitrate. The other isolates made small changes either upward or downward from the initial pH with only one pronounced trend. All the isolates markedly lowered the pH of the ammonium nitrate medium. However, this was not reflected in the growth of the isolate, as seen in Fig. 8, where the mycelial growth on media containing ammonium nitrate and sodium nitrate was somewhat similar.

These studies indicate that the four charcoal rot isolates are able to grow under a wide range of cultural conditions. Differences in the growth of the isolates were primarily differences in growth rates. In general the isolate from sugar beet grew the most vigorously, whereas the isolate from sorghum was the least vigorous. For the most part the four isolates showed similar growth curves, the greatest differences being in the pH test.

Table 2.—*Changes brought about in the pH of a synthetic agar medium by M. phaseoli*

Nitrogen source	Initial pH	pH after 3 days' growth				pH after uninoc. agar stood 2 weeks
		Sorghum isolate	Corn isolate	Catalpa isolate	Sugar beet isolate	
		CHANGES IN pH WITH DIFFERENT INITIAL H-ION CONCENTRATIONS				
Peptone	4.9	5.3	5.6	5.8	5.9	5.1
Peptone	6.0	5.9	6.1	6.2	6.2	6.1
Peptone	7.1	6.9	6.8	6.7	6.7	6.9
Peptone	8.0	7.5	6.9	7.3	7.6	7.6
		CHANGES IN pH WITH DIFFERENT SOURCES OF NITROGEN				
Peptone	7.0	6.3	6.7	6.6	7.1	6.6
Asparagine	6.9	6.4	7.2	7.2	7.5	6.7
Urea	7.0	6.6	6.6	6.5	7.1	6.8
Ammonium nitrate	6.9	4.5	4.8	5.5	4.3	6.6
Sodium nitrate	7.0	7.2	6.6	6.5	7.8	6.6
Calcium nitrate	7.0	6.9	6.8	6.7	7.9	6.8

The Effect of Soil Temperature and Soil Moisture on the Infection of the Grain Sorghum Variety Alliance by *M. Phaseoli*

Relation of Soil Temperature with High and Low Soil Moisture to Infection of Grain Sorghum Plants

Field surveys in 1940 and 1941 showed that charcoal stalk rot of sorghum was most serious in the south central portion of Nebraska. The annual precipitation, as shown in Table 3, was slightly less than that in the Panhandle where only a very small amount of charcoal rot was observed. However, the average daily temperatures during the summer months were much higher in the south central region than in the Panhandle, indicating that temperature was an important factor in the development of charcoal rot. This observation was in agreement with the results reported by Uppal et al. (*25*) who studied the effect of soil temperature and soil moisture on infection of sorghum in soil sterilized before inoculation. They grew the plants in soil temperature tanks of the Wisconsin type for 6 weeks, then removed the containers with the plants from the tanks and placed them in a greenhouse to mature. Stalk rot developed at 33.0°, 35.5°, and 37.5° with 35.5°C. as optimum. No rot developed above or below these soil temperatures.

High temperatures have also been found to favor the development of charcoal rot on other crops by several investigators. Taubenhaus (*21*) reports that the attack on sweet potatoes by *Rhizoctonia bataticola* (*M. phaseoli*) was favored by temperatures of 26.7° to 32.2°C. found in overheated houses during the sweating process and in warm storage bins, particularly near the stoves. Small (*18, 19*) found that beans were attacked by this fungus at high temperatures. Kendrick (*11*) made observa-

Table 3.—*The relationship of air temperature and rainfall to the occurrence of charcoal stalk rot in sorghum and corn in Nebraska*

Region	Year	Av. air temperature July, Aug., Sept.	Total rainfall		Disease severity
			July, Aug., Sept.	Jan. to July	
		Degrees C.	*In.*	*In.*	
South central	1940	24.0	4.8	9.1	severe
	1941	23.5	7.7	17.2	severe
	1944	22.5	10.6	20.0	slight
Panhandle	1940	21.7	2.8	6.3	very slight
	1941	20.0	4.5	11.4	none
	1944	20.0	4.8	9.6	none
Southeastern	1940	25.0	10.6	11.9	moderate
	1941	24.0	9.4	14.7	moderate
	1944	22.5	12.2	22.9	very slight

tions on beans in inoculated soils where the temperatures were recorded at the 1-inch and 3-inch depths. Only a few bean plants were infected when the daytime soil temperatures at the 1-inch and 3-inch levels were respectively, 22.2°–26.7° and 20.5°–25.0°C. However, nearly 100 per cent infection was obtained when the daytime soil temperatures were 36.7°–41.6°C. at a depth of 1 inch and 27.8°–31.7°C. at 3 inches. Kendrick concluded that charcoal rot of beans was favored by a daytime temperature of 35°C. or above in the surface inch of soil. Tompkins and Gardner (*24*) obtained the highest percentage of infection of Mexican Red bean seedlings at 31°, 34°, and 37°C. with generally lower percentages of infection at the lower temperatures. With cowpeas, on the other hand, very little infection was obtained at 34°, 37°, and 40°; but at 25° and 28°C. the cowpeas showed considerable infection. The authors suggest that the effect of temperature on the rate of growth of the seedlings may explain why the cowpeas were less severely infected at the higher temperatures. The cowpeas germinated vigorously at all temperatures while the beans germinated slowly at the higher temperatures. Tompkins (*23*) found that high temperatures also favored infection of sugar beets by *R. batatiçola.*

Uppal et al. (*25*) studied the effect of soil moisture on the development of charcoal rot and obtained no stalk rot at or below 50 per cent of the water-holding capacity of the soil.

No studies dealing with the inter-relationship of moisture and temperature and their effect on the development of charcoal rot have been made. Table 3 shows the severity of charcoal rot in three sections of Nebraska with the air temperature and rainfall for the three summer months. The rainfall from January through June is also shown because of the effect on the amount of moisture stored in the soil up to the first of July. The data indicate that very little charcoal rot occurs when the average air temperature during the summer growing season is 22.5°C. or below and the rainfall is 10.6 inches or above. Either low temperature or high moisture will retard or prevent the development of charcoal rot. Temperatures of 21.7°C. and lower prevented its occurrence in the Panhandle

even though rainfall was low. On the other hand, high soil moisture prevented the development of charcoal rot in irrigated fields in south central Nebraska during 1940 and 1941 with air temperatures averaging 23.5°C. and above, whereas in nonirrigated fields the disease was severe.

Because it was felt that more information was necessary to establish the relative importance of soil moisture and soil temperature, the following experiment was planned to determine two points—first, the effect of soil temperature on the infection of grain sorghum plants; and second, the effect of soil moisture on infection at different temperatures.

Method.—Two soil temperature tanks of the Wisconsin type, each holding eight soil cans of 10-kilogram capacity, were used at each of five temperatures, 25°, 28°, 31°, 34°, and 37°C. Temperatures were regulated by thermostatically controlled electrical heating coils with a variation of one degree above or below the recorded point. A soil mixture consisting of two parts of composted sod soil, two parts of sand, and one part of manure was inoculated by mixing approximately 600 cc. of an oat culture of *M. phaseoli* isolated from the grain sorghum variety Early Kalo with each 10 kilograms of soil. The moisture equivalent of the soil mixture was 12.9 per cent. The soil in eight cans at each temperature was kept at a moisture content of 18.6 per cent on an oven-dry basis, which was slightly above the optimum for growth; in an additional eight cans the soil moisture was kept at 9.7 per cent on an oven-dry basis, which was just above the wilting point. Two cans in each tank, one with the dry soil and one with the wet, were kept as uninoculated controls.

The soil was sterilized with steam for two hours at 15 pounds pressure and was then inoculated; ten kilograms were now placed in each can. Similar but uninoculated soil was used in the control cans.

The inoculum was prepared by placing 300 cc. of dry whole oats and 250 cc. of tap water in a quart milk bottle and sterilizing for two hours at 15 pounds steam pressure. A small disc of inoculum (mycelium and sclerotia) from a culture on potato-dextrose agar was added to each bottle and the oat culture allowed to incubate for 14 days at 23°C.

Several seeds of the Alliance variety of grain sorghum (Nebr. No. 33) were planted in each can and then thinned to two plants per can after seedling blight, caused by *M. phaseoli,* had ceased.

The temperature in the greenhouses was kept at approximately 32° in the day time and 24°C. at night.

Results.—The results of this test are presented in Table 4 and shown graphically in Fig. 10. There was a marked difference in the effect of temperature on seedling blight in the wet and dry soils. In the dry soil, blight was greatest at 37°C. and dropped off more or less gradually at each successively lower soil temperature to 25°C. A similar infection curve was obtained when the seedlings were grown in the wet soil, except at 25° where there was a higher percentage of seedling blight than at 28°C. Considerably more seedling blight occurred in the dry than in the wet soil at 37° and 34°C. It was about the same in both soils at 31°, and at 28° and 25°C. seedling death was greatest in the wet soil.

Table 4.—*Effect of soil temperature and soil moisture on the development of seedling blight and stalk rot of the sorghum variety Alliance*

Temperature	Soil treatment	Soil moisture	Seedling infection		Mature plant infection	
			Total seedlings emerged	Seedlings blighted	Total plants	Infected plants[1]
			No.	*%*	*No.*	*%*
37°C.	Inoc.	Wet	69	30.4	11	27.3
		Dry	41	43.9	14	64.3
	Check	Wet	14	14.3	4	0.0
		Dry	10	10.0	5	0.0
34°C.	Inoc.	Wet	70	25.7	14	21.4
		Dry	69	36.2	17	41.2
	Check	Wet	29	10.3	5	0.0
		Dry	15	6.7	6	0.0
31°C.	Inoc.	Wet	74	17.6	11	9.9
		Dry	56	17.9	16	18.8
	Check	Wet	15	13.3	4	0.0
		Dry	15	13.3	5	0.0
28°C.	Inoc.	Wet	38	13.2	7	0.0
		Dry	41	9.8	13	15.4
	Check	Wet	11	0.0	5	0.0
		Dry	16	6.3	6	0.0
25°C.	Inoc.	Wet	35	17.2	11	0.0
		Dry	36	5.6	14	0.0
	Check	Wet	24	4.2	6	0.0
		Dry	14	0.0	3	0.0

[1] Basal portion of stalk showing shredding, sclerotia, or both.

Stalk rot of the mature plants was also greatest at 37°C. with a decreasing amount at each lower soil temperature in both the wet and dry soils, Fig. 10. No stalk rot developed at either 28° or 25° in the wet soil or at 25°C. in the dry soil.

It is evident from this experiment that the development of both seedling blight and stalk rot was favored by the higher temperatures. A soil moisture of 9.7 per cent was more favorable for the development of stalk rot than 18.6 per cent. It was also more favorable for the development of seedling blight, except at the lower temperatures.

Stalks were considered to be infected with charcoal rot when sclerotia were present or when the stalk was badly shredded, with the pith at least 50 per cent disintegrated and the fibers free in the stalk. Healthy stalks either dried without disintegration of the pith or the stems frequently became hollow with the pith and fibrovascular strands closely attached to the inner surface of the stalk.

Only a small percentage of mature plants of the Alliance variety form sclerotia in the greenhouse. This percentage was greatly increased by allowing the stalks to thoroughly dry before harvest. Other stalks, which were soft and shredded at the base, developed sclerotia after digging when left at 32°C. for 10 to 20 days. Rarely did sclerotia develop on those plants that showed no disintegration or shredding at harvest.

Discoloration of the pith at the base of the stalk was common. Charcoal rot typically causes a black rot of green immature stalks; however,

in inoculation tests where several organisms were hypodermically injected into the base of the stem, the difference in the colors produced by the different organisms was so slight that it was not possible to use color as a diagnostic symptom.

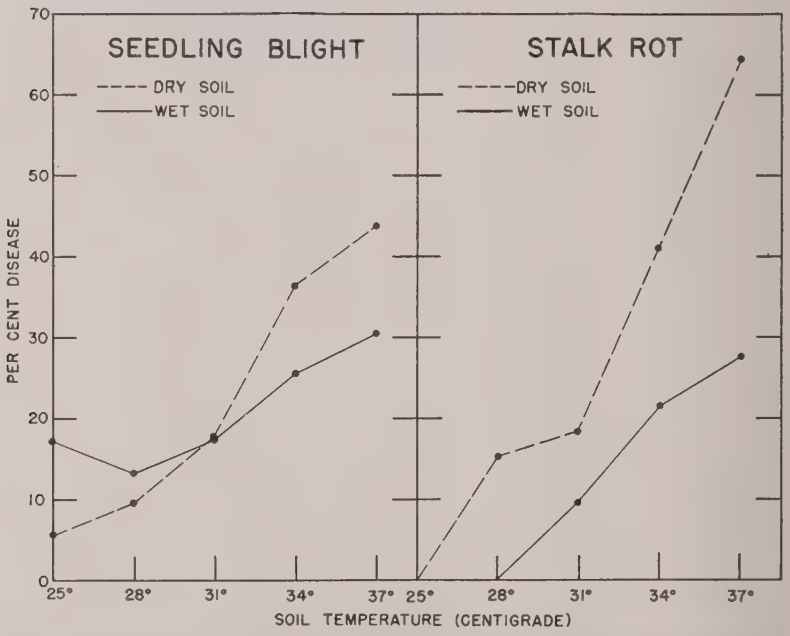

FIG. 10.—The effect of soil temperature on the development of seedling blight and stalk rot in wet and dry soils.

Relation of Soil Temperature to Infection of Grain Sorghum in Steam-Sterilized and Chloropicrin-Treated Soil at a Uniform Soil Moisture

The pathogenicity of an organism may be determined by growing plants in soil that was steam-sterilized before inoculation. The ability of an organism to produce disease in the field, however, depends in part on its ability to compete with other micro-organisms in the soil. In order to determine the virulence of *M. phaseoli* in competition with other soil organisms, an experiment was set up using steam-sterilized and chloropicrin-treated[1] soils in soil temperature tanks. The chloropicrin treatment probably destroyed many of the soil organisms but in view of the differences in plant growth, weed growth, and infection obtained, sterilization with steam was much more complete than with chloropicrin.

[1] The chloropicrin-treated soil cannot be considered to be the same as an untreated field soil; however, the treatment was necessary in this case to eliminate a troublesome insect.

Methods.—Five soil tanks each holding eight soil cans were used at each of four soil temperatures, 30°, 34°, 38°, and 42°C. Three cans of soil steamed for two hours at 15 pounds pressure and three cans of chloropicrin-treated soil were inoculated with *M. phaseoli* and placed in each tank. The chloropicrin-treated soil was treated with 2 cubic centimeters per hole in holes 12 inches apart in a soil mass 8 inches deep. One can of steam-treated and one can of chloropicrin-treated soil were left uninoculated as a check in four of the five tanks at each temperature. The moisture equivalent of the soil mixture was 12.9 per cent and the soil moisture was maintained at approximately 12 per cent on an oven-dry basis by weighing the cans periodically.

The soil was inoculated as previously described. Eight seeds of the grain sorghum variety Alliance were planted in each can and the plants thinned to two per can after they had reached a height of 8 inches.

Results.—The results were recorded on the basis of the number of seedlings emerged, the number of seedlings that died prior to thinning, expressed as seedling blight, and the number of mature plants that showed shredding of the basal portion of the stalk whether or not sclerotia were present.

The data in Table 5 show that there was a slight decrease in the number of seedlings emerged in all the inoculated soils over the check but the differences are probably significant only at 38° and 42°C. in the steam-sterilized soil.

Table 5.—*Effect of soil temperature and soil sterilization on infection of seedlings and mature plants of the sorghum variety Alliance*

Temperature	Soil treatment	Seeds planted	Seedlings emerged	Seedlings blighted	Mature plant infection	
					Total plants	Infected plants
		No.	*No.*	*%*	*No.*	*%*
			STEAM-STERILIZED SOIL			
42°C.	Inoc.	154	135	36.3	28	56.1
	Check	40	38	5.3	10	10.0
38°C.	Inoc.	150	127	29.1	29	79.3
	Check	40	40	5.0	10	10.0
34°C.	Inoc.	183	173	19.7	30	53.3
	Check	40	38	2.6	10	0.0
30°C.	Inoc.	178	169	7.7	30	30.0
	Check	40	38	0.0	10	0.0
			CHLOROPICRIN-TREATED SOIL			
42°C.	Inoc.	179	170	2.4	30	40.0
	Check	40	40	0.0	10	0.0
38°C.	Inoc.	161	153	0.0	41	51.2
	Check	40	40	0.0	10	0.0
34°C.	Inoc.	183	176	0.6	30	46.7
	Check	40	40	0.0	10	0.0
30°C.	Inoc.	196	190	0.5	30	36.7
	Check	40	40	0.0	10	0.0

There was no significant seedling blight at any temperature in the soil treated with chloropicrin and inoculated, as is shown in Fig. 11. In contrast, there was a significant loss of seedlings after emergence in the soil sterilized with steam before inoculation. The effect of temperature on seedling death in steam-sterilized soil was very similar to that shown in Table 4 with the greatest loss at the higher temperatures. Apparently *M. phaseoli* is an active parasite of sorghum seedlings in the absence of competing organisms but when it must compete with the microflora of field soil, it is relatively unimportant as the cause of seedling blight. These results agree with those reported by Semeniuk (*17*) with corn seedlings. Sanford (*15*), however, found that *Rhizoctonia solani* behaved quite differently and was much more virulent on potato stems in inoculated field soil than in the same soil steam sterilized and inoculated.

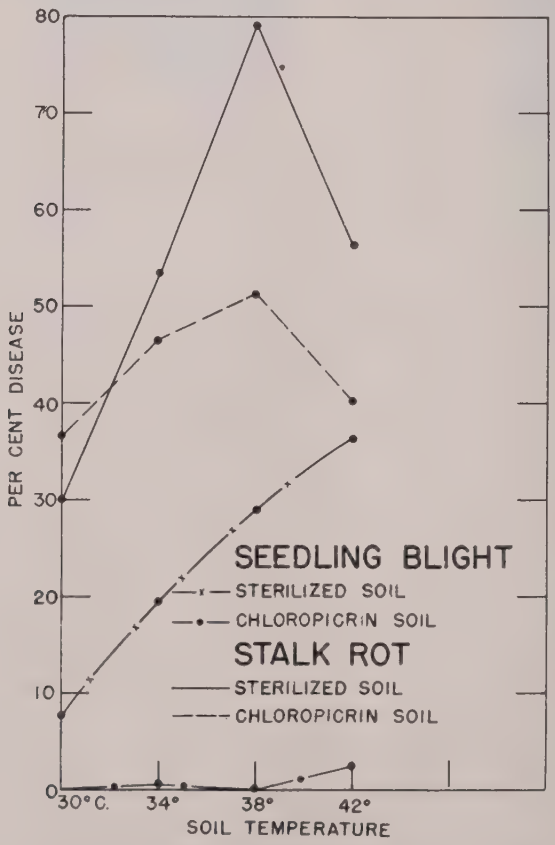

FIG. 11.—The effect of soil temperature on the development of seedling blight and stalk rot in steam-sterilized and chloropicrin-treated soils.

Infection of mature plants was a little higher in the steam-sterilized than in the chloropicrin-treated soil. However, the response to temperature was also very similar to that obtained in the previous test with 38°C. being the optimum for stalk rot development. Less infection of mature plants was obtained at 42° than at 38°C. This response may have been due to the poor growth made by the plants at 42°C. These plants were short with small stems and with small heads having most of the flowers blasted. During the last six weeks of growth they were very erect and showed rolled leaves as if suffering from a lack of moisture—even though the soil was moist. The check plants were only slightly larger than the plants in inoculated soil but they exhibited only slight leaf rolling. About 2 weeks before harvest the plants at 42°C. began dying from the top down, the leaves rolled and wilted, and the stem began drying while still green at the base. At harvest the root systems of these plants were severely rotted, with usually not more than one or two functioning roots.

The disease at temperatures of 38°C. and above was definitely a root rot of major importance pathologically, and the stem rotting and shredding with the formation of sclerotia occurred with few exceptions only with maturity and drying of the plant. In the few instances where the rot occurred in stems while still green, it could usually be detected as a wet, bluish-black discoloration of the stalk extending as high as 6 or 8 inches above the soil line. The interior of the stem was usually wet and brownish at first, later became black, and then finally as disintegration progressed the stalk became dry and sclerotia formed. There was considerable question as to how much of the discoloration of the interior of the stalk was due to the charcoal rot organism. Many plants were found with partial disintegration of the pith and abundant sclerotia on the pith and fibrovascular strands but with no discoloration of the pith other than that caused by the presence of the black sclerotia.

There was a marked difference in the height of plants in the steam-sterilized and chloropicrin-treated soil at the low temperatures with those in the steam-sterilized soil being the taller. The difference was about 8 inches at 30°C. and decreased gradually with each increase in temperature until at 42°C. there was no difference.

In an attempt more nearly to approach field soil conditions, another test was started in which the plants were grown in electrically heated soil beds containing a 6-inch layer of soil treated with chloropicrin before inoculation. Alliance grain sorghum seed was planted 3 inches apart in rows 15 inches apart. Plants were grown to maturity and the results were based on the presence or absence of rotting at the base of the stalk.

The results (Table 6) were similar to those shown in Tables 4 and 5, with the highest percentage of infection at 38° and the lowest infection at 32°C. Probably the most significant result obtained in this test was the high percentage of infected plants showing sclerotia at 38°C. The high soil temperature of 38°C. was the most favorable both for the production of stalk rot and the development of sclerotia in the plant.

Table 6.—*Relation of soil temperature to the infection of mature sorghum plants by M. phaseoli*

Soil temperature	Soil treatment	Total plants	Plants with stalk rot	
			Total	Plants with sclerotia
		No.	*%*	*%*
38°C.	Inoc.	94	39.4	25.5
	Check	90	0.0	0.0
34°C.	Inoc.	92	29.3	9.8
	Check	91	1.1	0.0
32°C.	Inoc.	96	21.9	2.1
	Check	97	0.0	0.0

Relation of Soil Moisture to Infection in Soil at a Uniform Temperature

Seedling infection

Previous experiments indicated that seedling blight was greatest at high temperatures in soils of low moisture content. In order to obtain more information at this point, an experiment was planned to determine the effect of soil moisture on seedling infection in soil sterilized before inoculation and kept at a uniform temperature.

Method.—A six-inch layer of inoculated soil with a moisture equivalent of 12.9 per cent was placed in greenhouse benches 6 ft. by 6 ft., heated from below by steam pipes. Three such benches were used, one with a moisture content on an oven-dry basis of approximately 10.2 per cent, just above the wilting point, one with a moisture content of approximately 14.6 per cent, and one with approximately 18.1 per cent moisture. Three similar benches with uninoculated soil were used as checks.

An attempt was made to control the air temperature in the greenhouse at 30°C.; however, this experiment was conducted in late spring and the air temperature frequently rose to 35° in the day time and occasionally to 40°C.

Table 7.—*Effect of soil moisture at a uniform temperature on infection of seedlings of the sorghum variety Alliance*

Soil moisture	Soil treatment	Seedlings emerged[1]		Healthy no symptoms	Seedlings blighted	Plants showing root rot when 10 in. tall[2]	Total percentage of infected plants
		No.	*%*	*%*	*%*	*%*	*%*
Dry[3]	Inoc.	335	51.5	10.5	42.1	47.5	89.6
	Check	24	48.0	91.7	8.3	0.0	8.3
Med.	Inoc.	551	84.8	43.2	13.1	43.7	56.8
	Check	45	90.0	93.3	0.0	6.7	6.7
Wet	Inoc.	583	89.7	66.6	2.1	31.4	33.5
	Check	48	96.0	100.0	0.0	0.0	0.0

[1] 650 seeds planted in each inoculated bench—50 seeds in each check bench.
[2] Seedlings escaping seedling blight were harvested after they had reached a height of approximately 10 inches.
[3] Dry = 10.2; Medium = 14.6; Wet = 18.1%.

The soil temperature 2 inches below the surface fluctuated between 33° and 38°, occasionally going as high as 42°C. It would have been desirable on the basis of the previous experiments, to have maintained the soil temperature between 38° and 42°C. but the equipment did not permit the maintenance of so high a temperature. The temperature in all the benches was uniformly the same.

Results.—The seedlings were carefully removed from the soil when they were 10 inches tall, and the results were derived from the number of seedlings that emerged, the number that died, and the number that did not die but showed infection of the primary and secondary roots. Previous experiments showed that no additional seedlings died from infection with *M. phaseoli* after they had reached a height of about 10 inches even though an examination revealed severe root infection of many plants. The results of the soil moisture study are shown in Table 7 and Fig. 12. The moisture content of the soil in the three benches was reflected in the emergence. Only a very slight effect on emergnce could be attributed to the organism and this only in the intermediate moisture and high moisture soils.

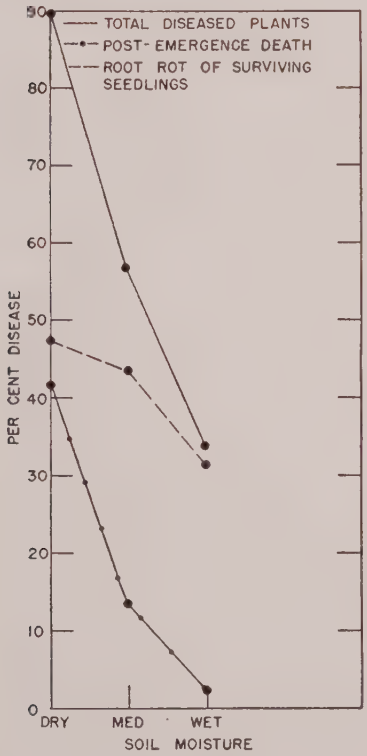

FIG. 12.—The effect of soil moisture on the development of seedling blight and on the development of root rot of seedlings that were not killed.

The greatest effect of moisture was on the seedling death. In the dry soil 42.1 per cent of the seedlings died after emergence, 13.1 per cent died in the soil kept at a medium moisture content, and only 2.1 per cent died in the wet soil. Only a small percentage of this loss in the dry soil could have been attributed to lack of moisture as shown by the checks and by the fact that many isolations on potato-dextrose agar from badly rotted roots yielded the charcoal rot organism. The roots of plants in the inoculated soil were brownish in color due to numerous lesions in marked contrast to the white roots of plants grown in uninoculated soil. There was also a higher percentage of surviving plants showing root rot in the dry than in the wet soil, although the difference was small in comparison to the difference in seedling death.

A direct relationship between the total percentage of diseased seedlings and the soil moisture is shown in Fig. 12. In the dry soil a high percentage of the seedlings was either killed or showed definite root lesions with the proportion being about the same. In the wet soil less than 50 per cent as much infection was obtained as in the dry, and most of this was a root rot with very few plants killed.

The ability of the seedlings to survive the attack of *M. phaseoli* was determined by whether or not the seedling had been weakened by the attack of the fungus to the extent that it was unable to put out adventitious or secondary roots. The results presented in Table 7 show that in the wet soil only 2.1 per cent of the seedlings died before the plants were dug yet an additional 31.4 per cent showed root lesions. The moisture content of the wet soil was sufficiently high that the seedlings were able to initiate and establish adventitious roots soon after emergence and apparently maintain normal growth. Infected adventitious roots seldom rotted completely off but showed many lesions varying in size and color, Fig. 3. In the dry soil, on the other hand, the seedlings grew much more slowly and a high percentage died before secondary roots could be established.

Mature plant infection

Stalk rot is the important phase of the charcoal rot disease in the field; thus it seemed desirable to study further the effect of soil moisture on the development of stalk rot and the production of sclerotia. Since sclerotia rarely appear until the plant matures and the stalk begins to dry, it was thought that varying the soil moisture supply during certain stages of growth might induce conditions that favor the development of sclerotia.

Composted soil consisting of three parts of soil and one part of manure was treated with chloropicrin and inoculated. The soil moisture was adjusted according to the schedule shown in Table 8 after the seedlings had emerged from the soil. The soil temperature in the benches fluctuated between 32° and 37°, averaging about 34°C. This was the highest average temperature that could be maintained under the conditions of this test. The air temperature was kept at 32° during the daytime and 27°C. at night.

Results.—Stalk rot of mature plants and the production of sclerotia, as shown in Table 8, were not as high as desired. However, the results are comparable to those shown in Table 6 at a soil temperature of 34°C. The highest percentage of stalk rot developed when the soil was kept dry during the time the grain was maturing. Dry periods during the early stages of growth were no more favorable for stalk rot development than continuously wet soil. In Soil A, which was kept wet throughout the experiment, eight plants in the inoculated soil and two plants in the uninoculated soil showed a wet rot at the base of the stalk. This rot was typical of the early stage of stalk infection; however, no shredding or formation of sclerotia occurred. In all the soils kept dry during the last seven weeks before harvest, the stalks of all of the diseased plants were shredded and some showed sclerotia.

When these results are considered in connection with those reported in Tables 4, 5, and 6, it may be seen that one of the problems in studying the stalk rot phase of charcoal rot in the greenhouse is to provide conditions that will encourage normal maturity of the stalk with the development of normal charcoal rot symptoms. A high percentage of root infection can be obtained, Table 7, but unless conditions are such that the stalks will mature and dry, typical stalk rot does not develop. Keeping the soil dry during the late stages of growth while the grain is maturing induces drying of the stalk and encourages stalk rot development, Table 8. Long dry periods prior to that time apparently have very little effect. No attempt has been made to determine the effect of alternating wet and dry periods of short duration.

Table 8.—*Relation of variable soil moisture to infection of mature plants of the sorghum variety Alliance by M. phaseoli*

Soil	Soil moisture				Soil treatment	Total plants	Plants with stalk rot	
	Seedling stage Oct. 11–Nov. 12	Growth stage Nov. 12–Dec. 13	Flowering stage Dec. 13–Jan. 25	Maturing stage Jan. 25–Mar. 13			Total	Plants with sclerotia
						No.	%	%
A[1]	Wet[2]	Wet	Wet	Wet	Inoc.	114	7.0	0.0
					Check	113	1.8	0.0
B	Wet	Wet	Wet	Dry[4]	Inoc.	107	27.1	6.5
					Check	102	3.9	0.0
C	Wet	Dry[3]	Wet	Dry[4]	Inoc.	115	16.5	7.8
D	Dry[3]	Wet	Wet	Dry[4]	Inoc.	104	25.9	12.5
					Check	101	3.9	0.0

[1] Planted October 11, 1943.
[2] The moisture content of the wet soil was maintained near 18.1 per cent.
[3] Soil moisture maintained near 10.2 per cent.
[4] No water applied during this period.

Variation in the Susceptibility of Sorghum Varieties to Charcoal Rot

Resistant varieties and selections offer the most promising control of charcoal rot. In a preliminary variety test Atlas, Western Blackhull, a selection from a cross of Kansas Orange by Dwarf Yellow Milo, and Alliance sorghum were planted in inoculated soil. The plants were grown in the greenhouse during the summer months of 1943 and pulled on the 25th of September. After being cut open and examined for rot and the presence of sclerotia, they were placed on a greenhouse bench for two weeks and again examined for sclerotia. There was a marked increase in the number of plants showing sclerotia during this period, Table 9. This increase in the number of plants showing sclerotia did not occur in some of the other tests however.

All of the varieties were quite susceptible, although Western Blackhull and Atlas were slightly less susceptible than the other two. The high percentage of stalk rot in Atlas was interesting in view of the fact that it has never shown stalk rot in the field in Nebraska.

Infection of the varieties obtained in the greenhouse during the summer of 1944 was not as high as in 1943. Western Blackhull showed less infection than any of the other varieties although still showing a high percentage of disease. More material is being tested in an effort to find parental material with sufficient resistance for breeding purposes.

There was a marked varietal difference in the percentage of diseased plants that showed sclerotia, Table 9. Western Blackhull showed the

Table 9.—*Infection of sorghum varieties by M. phaseoli*

Variety	Total plants	Infected plants	Diseased plants with sclerotia	
			Sept. 25	Oct. 8
	No.	*%*	*%*	*%*
		1943 TEST		
Alliance	37	94.6	70.3	73.0
Atlas	32	81.2	25.0	71.9
Western Blackhull	36	69.5	36.1	55.6
Kansas Orange x Dwarf Yellow Milo (Ks.Sel.24–136)	34	100.0	55.9	73.5
		1944 TEST		
Alliance	33	60.6	30.0	
Western Blackhull	34	29.4	70.0	
Plainsman	32	65.6	52.4	
Martin	36	75.0	48.3	
Club	30	86.7	57.7	
Western Blackhull x Day (Nebr.45)	35	65.7	43.5	
Midland	23	56.5	23.1	
Day	26	50.0	38.5	
Westland	29	75.9	45.4	

lowest percentage of diseased plants but a higher percentage of these had sclerotia than with any other variety. Club, on the other hand, showed the highest percentage of diseased plants with a high percentage of these having sclerotia. Sclerotia formed readily in the Alliance variety in the 1943 test but slowly in the 1944 test. This response emphasizes further the difficulty of obtaining sclerotia in the greenhouse where it is difficult to bring about normal plant ripening and drying. Of the varieties tested, Westland and Plainsman formed sclerotia quite readily and are highly susceptible. They appear to be the best varieties for greenhouse inoculation studies. In addition to being susceptible, they grow well in the greenhouse, they are short with a stalk of large diameter, and they develop a type of rot which approaches that to be found in the field.

Effect of Crop Rotation on the Development of Charcoal Rot

Crop rotation has long been recommended for the control of soil-borne diseases. No information is available, however, on the effect of crop rotation on the development of charcoal rot of sorghum and corn. In the field charcoal rot has been destructive in numerous rotation sequences,

and of especial interest was its occurrence in grain sorghum following three years of wheat inasmuch as the small grains, such as wheat, are not listed by Tehon and Boewe (*22*) or Mackie (*13*) as being susceptible to charcoal rot.

In order to obtain some information on the effect of short rotations on the development of charcoal rot in sorghum, an area in which the grain sorghum variety Alliance was severely affected in 1941 was selected and laid out in blocks 140 feet by 14 feet. Barley, oats, sweet clover, field beans, soybeans, corn, and sorghum were grown in these blocks without replication for two years and in the third year one-half of each block was planted to Early Hegari and the other half to Western Blackhull. Stalk rot was fairly uniform in all the plots planted to Early Hegari but there was very little disease in Western Blackhull in any of the blocks. Short rotations, therefore, do not offer much promise as a control measure.

The effect of long rotations on charcoal rot has not been determined; however, intervals of 6 or 7 years between crops of sorghum and corn in the nonirrigated regions of Nebraska where these crops are adapted is not practical under the present cropping system.

Method of Infection

Macroscopic observations indicated that infection often took place either at the base or the tip of sorghum roots. There was some question, however, whether the infection of the mesocotyl and the base of the large secondary roots was the result of the fungus following up a small feeding root or whether it was by direct penetration of the epidermis. Kendrick (*11*) concluded that this organism could probably penetrate the unwounded epidermis of a bean stem through the primordial tissue in the axis of the cotyledon. He found no wounds or natural openings in longitudinal and cross sections cut through the hypocotyl and attached cotyledon of healthy and infected seedlings. The stem tissue at the base of the small cotyledonary petiole was invaded first, then the fungus advanced into the cotyledon.

In the case of root infection by *M. phaseoli*, there seems to be general agreement that the charcoal rot fungus enters the plant through the small feeding roots and progresses toward the larger roots and the crown (*3, 5, 14*).

The invasion of the sorghum root tissues was studied in stained microtome and fresh sections. The mycelium was found to be mostly intercellular and parallel to the main axis of the root. Growth occurred first in the cortical tissues, and later the inner tissues were invaded. Kendrick (*11*) observed that bean stems were invaded in a similar manner. He found, by means of stained microtome sections, that the fungus was intercellular and confined in the early stages to the cortical tissues. Growth of the fungus was more rapid longitudinally through the cortical tissues than radially or tangentially.

Rot of the stem tissues of sorghum usually does not occur until late in the growth of the plant. It is often possible in immature plants to observe a root rotted right up to the point of transition into stem tissue.

There the progress of the rot has stopped abruptly. However, as the plant approaches maturity the stem tissue apparently becomes more susceptible to the rotting action of the fungus.

It is not known how long the fungus may be present in the tissue at the base of the sorghum stem before rotting occurs. Weimer (*26*) made isolations from root and stem tissue of seedlings of *Chamaecrista procumbens* showing little or no evidence of infection and obtained the organism from the above-ground stem of over 40 per cent of the plants. It is thus possible that the mycelium of *M. phaseoli* may be present in the stem tissue of the sorghum plant sometime before there is any visible rot.

Discussion

The charcoal rot producing organism, *Macrophomina phaseoli*, is capable of causing a seedling blight and also a basal stalk rot as the plant approaches maturity. The seedling blight is greatly influenced by soil moisture and soil temperature, conditions that affect both the growth of the host and the growth of the organism.

A high soil temperature of at least 35° favored the growth of the organism and a soil temperature of 42°C. was most favorable for the production of seedling blight. The development of stalk rot of mature plants was also favored by high soil temperatures with the highest percentage of rot developing at 38°C. Above that temperature plant development in the soil temperature tanks was so reduced that there was some question whether the reduced stalk rot was a result of the abnormal maturity of the plant or a reduction in the activity of the organism at the high temperature. The development of maximum charcoal rot at high soil temperatures is probably a result of the effect of temperature on both the organism and the host. High temperatures are unfavorable for the best growth of the host but are favorable for the growth of the organism, as shown by this study and that of Uppal et al. (*25*) and Kendrick (*11*). A similar situation was encountered by Tompkins and Gardner (*24*) who found that charcoal rot infection of beans was greatest at the higher temperatures which were unfavorable for the germination and growth of the seedling.

Low soil moisture retards the growth of the host and favors the development of seedling blight and stalk rot. In greenhouse tests the seedlings that were the slowest emerging, particularly in the dry soils, were much more likely to rot off than those that emerged rapidly. The seedlings that emerged rapidly, grew more vigorously, established secondary roots, and managed to live in spite of the many root lesions, whereas plants that emerged slowly usually were killed before they were able to establish secondary roots.

The effect of moisture on the development of stalk rot would seem to be primarily an effect on the host. When moisture is sufficiently abundant that the host grows continuously right up to harvest the stalk rot phase of the disease is kept at a minimum. In all instances the highest percentage of stalk rot was obtained in the low moisture series. According to the results in Table 8, low soil moisture during the latter part of the

growing season is the most favorable for the development of stalk rot.

The results of the soil moisture and temperature studies help to explain the appearance and distribution of charcoal stalk rot in the field. Either moisture or temperature can be the factor limiting its distribution and severity. The greatest damage from charcoal stalk rot in Nebraska occurred in the south central counties in 1940 when the total rainfall during July, August, and September was 4.8 inches and the average daily air temperature was 24°C., Table 3. The average maximum daily temperatures during the hottest months, July and August, varied from 30° to 36°C. with the daily temperatures often going over 38°C. Even with these high temperatures charcoal stalk rot has been very difficult to find in irrigated fields where the moisture supply was ample throughout the season. It has also been relatively hard to find in low areas in fields in eastern Nebraska where the rainfall during the summer months since charcoal rot was first observed has been 9.4 inches or more. However, it has often been found in corn and sorghum growing on hilly land, particularly near the "shoulder" of a hill where the best drainage occurred. In western Nebraska where the average summer rainfall has been 4.8 inches or less, charcoal rot has been unimportant; however, the average air temperature has been below 22.5°C., which was the minimum average air temperature at which stalk rot occurred in south central and southeastern Nebraska.

Apparently the microflora of the soil greatly retarded the activity of the charcoal rot organism. Practically no seedling blight occurred in chloropicrin-treated and inoculated soil, whereas a high percentage of seedlings were killed in soil steam-sterilized and inoculated. In mature plants the effect was not so great although the percentage of stalk rot in the steam-sterilized and inoculated soil was still significantly higher than in the chloropicrin-treated soil at the higher temperatures. Semeniuk (*17*) obtained similar results with corn seedlings, reporting that no disease developed in nonsteamed inoculated soil.

The ability of one organism to suppress the virulence of another has been noted by numerous workers and reviewed by Garrard and Lockhead (*8*) and Sanford (*15*). Broadfoot (*4*) found that *Ophiobolus graminis* Sacc. was extremely virulent in steam-sterilized soil but its virulence was decreased by 77 per cent when the soil was exposed to recontamination for 10 days before being planted. Henry (*10*) and Sanford and Broadfoot (*16*) found that *O. graminis*, *Helminthosporium sativum* P. B. & K. and *Fusarium culmorum* (W. G. Sm.) Sacc. lost their virulence rapidly when placed in field soil. Tests of many isolates showed that some organisms were more effective than others in suppressing growth of a certain organism. On the other hand, Sanford (*15*) and Blair (*2*) found that *Rhizoctonia solani* Kühn was more virulent when mixed in field soil.

Seedling infection of the grain sorghum variety Alliance by *M. phaseoli* followed the pattern shown by *O. graminis*, *H. sativum*, and *F. culmorum* in that severe infection occurred in soil that had been steam sterilized but very little occurred in soil that had not been sterilized. Infection of mature plants, however, occurred in both soils but infection was higher

in the soil that had been steam sterilized before inoculation.

The nature of this biological action has been discussed by Garrett (7) who states that the effect of soil organisms on the activity of *O. graminis* on the roots of the growing wheat crop is that of inhibition. The respiration of the soil organisms increases the carbon dioxide concentration along the root which inhibits the growth of *O. graminis.* With the resting mycelium of *O. graminis,* however, the effect is that of competition for the available nutrients, especially nitrogen.

The charcoal rot organism enters the sorghum plant through the roots and progresses into the stem producing the stalk rot. The advance of the organism is primarily through the cortical tissues, and rot development may be extensive in these tissues before the stele is invaded. Death of the root does not occur until the inner tissues are destroyed.

SUMMARY

1. Charcoal rot causes a serious stalk rot of corn and sorghum in Nebraska.

2. In culture the organism prefers high temperatures of at least 35°C., grows well on a large number of nitrogen and carbohydrate compounds, and grows well over a pH range of 5.0 to 8.0.

3. Either low soil temperature or high soil moisture throughout the growing season prevents the occurrence of charcoal rot in Nebraska.

4. A soil temperature of 42°C. was most favorable for the development of seedling blight, whereas a soil temperature of 38°C. was most favorable for the development of stalk rot of mature plants in greenhouse studies.

5. Seedling blight was favored by low soil moisture. Very little seedling blight occurred in soils with a high moisture content although many seedlings had infected roots.

6. Stalk rot of mature plants was much greater in dry than in wet soils. Low soil moisture just preceding and during maturity was essential for the development of a high percentage of rotted stalks and the formation of sclerotia.

7. Seedling blight was very low in nonsterile inoculated soil but high in soil steam-sterilized before inoculation.

8. Susceptibility of sorghum varieties to *M. phaseoli* in inoculated soil varied considerably.

9. Growth of the fungus mycelium in the root was intercellular and confined in the early stages to the cortical tissues.

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